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Genome Note

Draft genome sequence of *Enterococcus faecium* E86, a strain producing broad-spectrum antimicrobial peptides: Description of a novel bacteriocin immunity protein and a novel sequence type

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ABSTRACT

Objectives: The aim of this study was to report the draft genome sequence of the bacteriocinogenic strain *Enterococcus faecium* E86. Bacteriocins are prokaryotic peptides or proteins with antimicrobial activity. The genome information may contribute to the identification of enterocins produced by this strain that exhibit inhibitory activity against the foodborne pathogen *Listeria monocytogenes* and vancomycin-resistant enterococci (VRE) involved in human infections, among other bacterial genera and species.
Methods: An Illumina MiSeq platform was used for genome sequencing. De novo assembly of 5 735 838 paired-end reads was done using the A5-miseq pipeline, yielding >300-fold average genome coverage. Genome annotation was performed by the RAST server, and mining of the bacteriocinogenic gene clusters was done using the BAGEL3 and antiSMASH v.4 platforms.
Results: The total scaffold size was determined to be 2 689 107 bp, approximately 2.7 Mbp, featuring a G + C content of 38.1%. The genome contains 2858 coding sequences and 74 RNA genes. Genome analyses revealed the presence of: 30 genes involved in drug resistance; 2 bacteriocinogenic gene clusters (for enterocin P and enterocin TW21); EntiTW21, a novel bacteriocin immunity protein and a novel multilocus sequence type (ST1500).
Conclusion: This work highlights the potential biotechnological application of this strain for the production of enterocin P, a bacteriocin that can be employed in the food industry as a biopreservative against *L. monocytogenes* and as an alternative to classical antibiotics against VRE.

1. Introduction

Enterococcus is a genus that belongs to the Enterococcaceae family, being classified as a member of the group of micro-organisms known as lactic acid bacteria. The genus *Enterococcus* is currently composed of 38 species that differ in motility, pigment production and capacity to produce acid from various carbohydrate sources [1]. These bacteria are Gram-positive, facultative anaerobe and oxidase-negative cocci that do not produce spores, grow at high NaCl concentrations (up to 6.5%) and occur generally either in pairs or in short chains [2]. Enterococci have the ability to produce various types of substances with biotechnological potential, such as enzymes, lactic acid, aromatic compounds and bacteriocins, among others [3]. Bacteriocins are ribosomally-synthesised antimicrobial peptides or proteins produced by prokaryotes [4]. Enterocins, which are bacteriocins produced by enterococci, generally have the ability to inhibit *Listeria monocytogenes*, an important foodborne pathogen [4], as well as other bacterial pathogens [5]. In the present work, the draft genome of *Enterococcus faecium* E86, a strain isolated from meat pie, is presented. This strain was shown to exhibit inhibitory activity against strains of *Pediococcus* spp., *Lactobacillus* spp., *Listeria* spp.

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(including *L. monocytogenes*) and vancomycin-resistant enterococci (VRE) [5].

2. Methods

Genomic DNA of *E. faecium* E86 was extracted using a GenElute™ Bacterial Genomic DNA Kit (Sigma-Aldrich, St Louis, MO) according to the manufacturer's instructions. A DNA library was then prepared with the extracted DNA using a Nextera XT DNA Library Preparation Kit (Illumina Inc., San Diego, CA). Whole-genome shotgun sequencing was performed on an Illumina MiSeq system (Illumina Inc.). De novo assembly of 5 735 838 paired-end reads was done using the A5-miseq pipeline (https://sourceforge.net/projects/ngopt/). Genome annotation was performed using the Rapid Annotation using Subsystem Technology (RAST) server (http://rast.nmpdr.org). Mining of bacteriocinogenic gene clusters was done using antiSMASH v.4 (https://antismash.secondarymetabolites.org) and BAGEL3 (http://bagel.molgenrug.nl) platforms as well as by visual inspection. The BLASTP program was used to search for sequence similarities between the predicted amino acid sequences of each open reading frame (ORF) of the bacteriocinogenic gene clusters and other proteins available in GenBank. Identification of putative phages inserted in the genome was done using the PHAST server (http://phast.wishartlab.com). The multilocus sequence type (MLST) was determined by the PubMLST platform (https://pubmlst.org).

3. Results

Owing to its biotechnological potential for the control of important human pathogens, here we report the draft genome of *E. faecium* E86, a strain isolated from meat pie. The resulting draft genome exceeded 300-fold coverage and consisted of 83 scaffolds ranging from 507 bp to 241 631 bp, with an N_{50} value estimated at 86 854 bp.

The total scaffold size was determined to be 2 689 107 bp, approximately 2.7 Mbp, featuring a G+C content of 38.1%. The genome contains 2858 coding sequences and 74 RNA genes.

The genome of *E. faecium* E86 was shown to contain two complete gene clusters encoding enterocin P (*entPentiP*) [6] and enterocin TW21 (*entTW21entiTW21*) [7] (Fig. 1A,B) in scaffold 41. The enterocin P cluster found was to be almost identical (>99% identity) to that described by Cintas et al. [6]. Regarding the enterocin TW21 cluster, the structural gene, which shows >99% identity to that described by Chang et al. [7], appears to present a base pair deletion in position 550 of scaffold 41, which would lead to a premature termination codon (TGA) and translation of an inactive core peptide. The gene encoding the novel putative immunity protein EntiTW21 (Fig. 1C) is located downstream of the *entTW21* gene, being preceded by a putative ribosomal binding site (RBS) and a putative Sig A-dependent promoter, suggesting that it may be transcribed independently from the bacteriocin structural gene. Scaffold 41 (14 855 bp), where both bacteriocinogenic gene clusters were found, shows a high level of identity (99.8%) with the 49-kb plasmid pGR17 (GenBank accession no. CP033377.1). Such data suggest that both enterocin gene clusters may be encoded by the same plasmid DNA.

The genome annotation also reported 30 genes involved in drug resistance, such as genes related to heavy metal (mercury and cadmium) and antimicrobial resistance (fluoroquinolone resistance, β-lactam resistance and multidrug-resistance efflux pumps) as well as two virulence factor genes, namely *acm*, an adhesin for collagen, and *efaA*^{fm}, a gelatinase. In addition, the bacterium carries an intact prophage in its genome: *Streptococcus* phage phiARI0468-1 (**NC_031915**).

The MLST type found was shown to be very similar to ST96 with a substitution of adenine for guanine at position 81 in the *pstS* gene. This mutation indicates a novel allele *pstS* 138 and, consequently, a novel MLST sequence type, which was designated as ST1500. The alignment of both bacteriocin gene clusters with the NCBI genome databank revealed that their presence is not related to ST96, since no strain whose genome exhibits a high-level identity to them belongs to ST96. They were found in strains belonging to ST17, ST21, ST78, ST80, ST117, ST456, ST736, ST796, ST812 and ST869.

4. Discussion

The draft genome of *E. faecium* E86 reinforces that the *Enterococcus* genus is an important reservoir of genes encoding bacteriocins, which may exhibit broad-spectrum activity against human pathogens, as well as drug resistance. It also provides valuable information on the enterocins responsible for its antagonistic activity. The enterocin P bacteriocinogenic cluster has been well known for 20 years [7]. However, up to now, only the

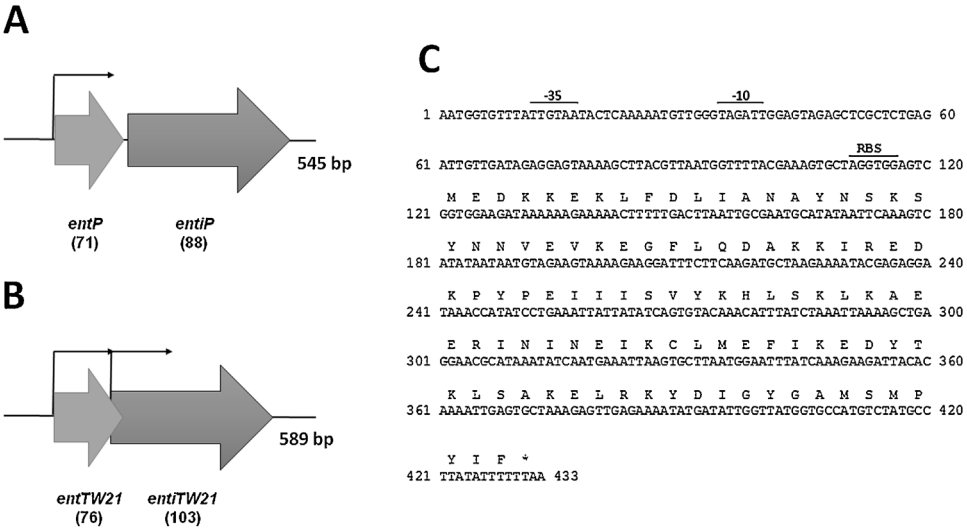

Fig. 1. Representation of the gene clusters of (A) enterocin P and (B) enterocin TW21. The number under the genes indicates the number of amino acids of each gene product. The arrows indicate putative promoters. (C) Nucleotide sequence of the gene encoding the putative enterocin TW21 immunity protein. The deduced amino acid sequence is shown under the DNA sequence. The –10 and –35 regions of the putative Sig A-dependent promoter and the ribosomal binding site (RBS) are underlined.

enterocin TW21 structural gene had been sequenced [6]. The complete enterocin TW21 gene cluster is now described owing to the identification of its immunity protein, EntiTW21, never reported before. As export of enterocin TW21 appears to depend on the bacterial major secretory pathway Sec, no other genes appear to be required for its production. As the structural gene encoding enterocin TW21 appears to be non-functional due to a nonsense mutation, the draft genome of *E. faecium* E86 highlights the potential application of enterocin P as an alternative agent to the classical antibiotics against VRE in human medicine and as a biopreservative against *L. monocytogenes* in the food industry. Moreover, as both enterocin gene clusters were found in strains belonging to different STs, these results suggest their presence in mobile genetic elements that must have been acquired by horizontal gene transfer.

Nucleotide sequence accession no

This Whole Genome Shotgun project has been deposited at DBJ/ENA/GenBank under the accession no. SIHT00000000. The version described in this paper is version SIHT01000000.

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Competing interests

None declared.

Ethical approval

Not required.

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